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Translation
Attached

权利要求书 1 页 说明书 7 页

[54] 发明名称 盐酸伐昔洛韦冻干制剂及其制备方法

[57] 摘要

本发明提供了一种盐酸伐昔洛韦冻干制剂及其制备方法，该冻干制剂是由活性成分盐酸伐昔洛韦与药学上可接受的赋型剂均匀混合组成的；盐酸伐昔洛韦与所述赋型剂按重量计用量比为 100－500：0－500。该盐酸伐昔洛韦的冻干制剂避免了口服制剂的一些不足，减少了口服制剂对胃肠道的刺激，增加制剂多样化，方便临床用药，提高生物利用度，特别是给急性患者用药提供了更好的选择。

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1、一种盐酸伐昔洛韦冻干制剂，其特征在于，该冻干制剂是由活性成分盐酸伐昔洛韦与药学上可接受的赋型剂均匀混合组成的；盐酸伐昔洛韦与所述赋型剂按重量计用量比为 75-500：0-500。

2、如权利要求 1 所述的盐酸伐昔洛韦冻干制剂，其中盐酸伐昔洛韦与所述赋型剂按重量计用量比为 80-500：10-500。

3、如权利要求 2 所述的盐酸伐昔洛韦冻干制剂，其中盐酸伐昔洛韦与所述赋型剂按重量计用量比为 90-500：20-500。

4、如权利要求 3 所述的盐酸伐昔洛韦冻干制剂，其中盐酸伐昔洛韦与所述赋型剂按重量计用量比为 100-500：25-500。

5、如权利要求 1 所述的盐酸伐昔洛韦冻干制剂，其中所述的药学上可接受的赋型剂选自以下物质中的一种或多种：甘露醇、山梨醇、氯化钠、葡萄糖、蔗糖、乳糖、右旋糖苷。

6、如权利要求 1-5 之任一所述的盐酸伐昔洛韦冻干制剂，其中所述的冻干制剂中还包括稳定剂、镇痛剂和/或缓冲剂。

7、如权利要求 1 所述的盐酸伐昔洛韦冻干制剂的制备方法，其特征在于，该方法包括以下步骤：取盐酸伐昔洛韦 75-500 重量份，药学上可接受的赋型剂 0-500 重量份溶解于注射用水中，用 pH 值调节剂调节该溶液 pH 值至 5.0-8.5 后，将该溶液冷冻干燥。

8、如权利要求 7 所述的制备方法，其中所述的药学上可接受的赋型剂选自以下物质中的一种或多种：甘露醇、山梨醇、氯化钠、葡萄糖、蔗糖、乳糖、右旋糖苷。

9、如权利要求 7 所述的制备方法，其中所述的 pH 值调节剂选自由以下物质中的几种组成的缓冲溶液：氢氧化钠，氢氧化钾，氨水，磷酸，柠檬酸，醋酸，乳酸及其盐。

10、如权利要求 7-9 之任一所述的制备方法，其中所述的溶液中还包括稳定剂、镇痛剂和/或缓冲剂。

盐酸伐昔洛韦冻干制剂及其制备方法

技术领域

本发明涉及一种冻干制剂及其制备方法，具体来说，本发明涉及一种盐酸伐昔洛韦冻干制剂及其制备方法。

背景技术

盐酸伐昔洛韦是阿昔洛韦的前体药物，是阿昔洛韦与缬氨酸形成酯的盐酸盐，分子式为：$C_{13}H_{20}N_6O_4 \cdot HCL$，盐酸伐昔洛韦口服后迅速转化为阿昔洛韦，在病毒感染细胞内，被脱氧苷激酶活化，进一步磷酸化为三磷酸酯，通过抑制 DNA 聚合酶，并在 DNA 聚合酶作用下，与增长的 DNA 结合，终止病毒的复制，从而达到抗病毒作用。临床上用于带状疱疹、生殖器疱疹、乙肝、尖锐湿疣等的治疗，并广泛的用于其他疱疹病毒感染的治疗。伐昔洛韦 1995 年 1 月在英国和爱尔兰上市，同年 6 月获得美国 FDA 批准，现已在数十个国家用于临床。作为一个新型的核苷类抗病毒药，我国于 1996 年正式获卫生部批准。（青海医药杂志 2001 年第 31 卷第 4 期）

目前，关于盐酸伐昔洛韦的制剂报道很少，国内未见相关专利文献报道，且国内外只有口服制剂，未见有关注射剂的报道，为了提高疗效，为医生用药提供更多的选择，特别是对急性疾病的预防与治疗提供更多的选择，发明一种注射剂是必要的。由于冻干制剂稳定性好、使用方便、便于运输及储存。所以，发明了盐酸伐昔洛韦冻干制剂的制备方法。

发明内容

针对盐酸伐昔洛韦口服制剂起效慢、长期用药对胃肠道刺激性大的缺点。本发明提供了一种盐酸伐昔洛韦冻干制剂及其制备方法。

为克服上述缺点，本发明采用如下技术方案：

本发明提供了一种盐酸伐昔洛韦冻干制剂，其特征在于，该冻干

制剂是由活性成分盐酸伐昔洛韦与药学上可接受的赋型剂均匀混合组成的;盐酸伐昔洛韦与所述赋型剂按重量计用量比为 75-500:0-500。其中，盐酸伐昔洛韦与所述赋型剂按重量计用量比优选为 80-500:10-500，更优选为 90-500: 20-500，最优选为 100-500: 25-500。

其中所述的药学上可接受的赋型剂选自以下物质中的一种或多种：甘露醇、山梨醇、氯化钠、葡萄糖、蔗糖、乳糖、右旋糖苷。

另外，所述的冻干制剂中还包括稳定剂、镇痛剂和/或缓冲剂

此外，本发明还提供了一种盐酸伐昔洛韦冻干制剂的制备方法，其特征在于，该方法包括以下步骤：取盐酸伐昔洛韦 75-500 重量份，药学上可接受的赋型剂 0-500 重量份溶解于注射用水中，加入 0.1%-1.0%（w/v）的针用活性炭，水浴加热下搅拌 20-30 分钟，放至室温，过滤脱炭，调 pH 值至 5.0-8.5 ，以 0.22μm 微孔滤膜过滤除菌，测定含量。将溶液在无菌条件下灌装入西林瓶中，冷冻干燥。

其中所述的药学上可接受的赋型剂选自以下物质中的一种或多种：甘露醇、山梨醇、氯化钠、葡萄糖、蔗糖、乳糖、右旋糖苷；其中所述的 pH 值调节剂选自由以下物质中的几种组成的缓冲溶液：氢氧化钠，氢氧化钾，氨水，磷酸，柠檬酸，醋酸，乳酸及其盐。其中所述的磷酸盐包括磷酸钾、磷酸钠、磷酸一氢钠、磷酸二氢钠、磷酸二氢钾等。

进一步来说，其中所述的溶液中还包括稳定剂、镇痛剂和/或缓冲剂。如，亚硫酸盐类、抗坏血酸、硫脲、半胱氨酸、抗坏血酸棕榈酸酯、维生素 E、依地酸钙钠、依地酸钙二钠、盐酸普鲁卡因、盐酸利多卡因、氢氧化钠，氢氧化钾，氨水，磷酸盐，柠檬酸盐，醋酸盐，乳酸盐等 。其中所述的磷酸盐包括磷酸钾、磷酸钠、磷酸一氢钠、磷酸二氢钠、磷酸二氢钾等。

更具体来说，本发明所提供的制备方法包括以下步骤：

（1）取赋型剂加入到注射用水中搅拌溶解。

（2）在第（1）步骤所得的溶液中加入 0.1%-1.0%（w/v）的针用

活性炭，水浴加热下搅拌 20-30 分钟，放至室温，过滤脱炭，调 pH 值至 5.0-8.5 ，以 0.22μm 微孔滤膜过滤除菌，测定含量。

（3）将溶液在无菌条件下灌装入西林瓶中。

（4）预先分别将冻干箱内得温度降至-40℃以下，将样品置于冷冻箱中，待样品冷至-35℃后，冷冻 2 小时，然后抽真空并在 15-30 小时内逐渐升温至室温。20℃-30℃再干燥 2 小时。

临床应用时加注射用溶媒（注射用生理盐水或葡萄糖注射液）溶解，摇匀使用。

采用本发明所提供的盐酸伐昔洛韦冻干制剂的制备方法，工艺重现性好（连续制备三批样品，均达到质量要求），成品率高（三批样品的成品率均大于 98.0%）。所制备出的冻干制剂有优良的外观，优良的复溶性和保存稳定性，与葡萄糖及氯化钠注射液配伍稳定，溶液澄明度良好。产品质量稳定，使用方便，即可用于肌肉注射也可用于静脉输注，同时避免了口服制剂的一些不足，增加制剂多样化，方便临床用药，提高生物利用度，特别是给急性患者用药提供了更好的选择。

具体实施方式

实施例 1:

取盐酸伐昔洛韦 100mg 及甘露醇 50mg，加入到 1ML 注射用水中，搅拌使其溶解，按照 0.5%（w/v）的用量加入活性炭，20℃搅拌 40 分钟，脱炭过滤，加入氢氧化钠调溶液的 pH 值至 8.5，所得滤液用 0.22μm 微孔滤膜过滤除菌，测定含量。在无菌条件下将所得溶液装入西林瓶中，置冷冻干燥机中冷冻干燥 20 小时后，轧盖，出箱，包装即得本发明的盐酸伐昔洛韦冻干制剂。

为了考察本发明冻干制剂处方工艺的适应性及稳定性，对上述冻干制剂进行了强光（4500±500Lx）、高温（60℃）、高湿（相对湿度 90±5%）考察，其结果如下表:

影响因素	放置时间（天）	外观色泽	有关物质（%）	澄明度	含量（%）
强光	0	白色疏松快状物	合格	合格	99.3
	5	白色疏松快状物	合格	合格	99.3
	10	白色疏松快状物	合格	合格	98.9
高温	0	白色疏松快状物	合格	合格	101.0
	5	白色疏松快状物	合格	合格	98.9
	10	白色疏松快状物	合格	合格	99.2
高湿	0	白色疏松快状物	合格	合格	99.5
	5	白色疏松快状物	合格	合格	100.2
	10	白色疏松快状物	合格	合格	98.8

以上试验结果表明，采用本发明制备的盐酸伐昔洛韦冻干制剂对强光、高温、高湿均质量稳定，在包装及贮存上无需特殊要求。

实施例 2:

取盐酸伐昔洛韦 200mg 及山梨醇 75mg，加入到 2ML 注射用水中，搅拌使其溶解，按照 0.5%（w/v）的用量加入活性炭，30℃搅拌 30 分钟，脱炭过滤，加入氢氧化钾调溶液的 pH 值至 8.0，所得滤液用 0.22μm 微孔滤膜过滤除菌，测定含量。在无菌条件下将所得溶液装入西林瓶中，放入温度为-20℃的冻干箱内进行预冻，再将冻干箱内真空度升至 13.33Pa 以下关闭冷冻机，通过搁置板下的加热系统缓缓加温，供给样品在升华干燥过程中所需的热量，使冻结样品的温度逐渐升高至约-20℃，药液中的水分就可升华，持续升华干燥 15 小时，直至水分除尽，当升华干燥完成后，为尽可能除去残余水分，需要进一步干燥，再干燥温度控制在 15℃。板温控制在 30℃以下，直至样品温度与板温相同，即达干燥终点。冷凝器温度达到－33℃，再干燥 11 小时，轧盖，出箱，包装即得本发明的盐酸伐昔洛韦冻干制剂。

实施例 3:

取盐酸伐昔洛韦 500mg 及氯化钠 150mg、葡萄糖 100 mg、蔗糖

200 mg、乳糖 50 mg，加入到 3ML 注射用水中，搅拌使其溶解，按照 1.0%（w/v）的用量加入活性炭，40℃搅拌 20 分钟，脱炭过滤，加入磷酸缓冲液调溶液的 pH 值至 5.0，所得滤液用 0.22μm 微孔滤膜过滤除菌，测定含量。在无菌条件下将所得溶液装入西林瓶中，放入温度为-25℃的冻干箱内进行预冻，再将冻干箱内真空度升至 13.33Pa 以下关闭冷冻机，通过搁置板下的加热系统缓缓加温，供给样品在升华干燥过程中所需的热量，使冻结样品的温度逐渐升高至约-20℃，药液中的水分就可升华，持续升华干燥 17 小时，直至水分除尽，当升华干燥完成后，为尽可能除去残余水分，需要进一步干燥，再干燥温度控制在 20℃。板温控制在 30℃以下，直至样品温度与板温相同，即达干燥终点。冷凝器温度达到－33℃，再干燥 10 小时，轧盖，出箱，包装即得本发明的盐酸伐昔洛韦冻干制剂。

实施例 4:

取盐酸伐昔洛韦 100mg 加入到 2.5ML 注射用水中，搅拌使其溶解，按照 1.0%（w/v）的用量加入活性炭，40℃搅拌 20 分钟，脱炭过滤，加入氨水调溶液的 pH 值至 7.5，所得滤液用 0.22μm 微孔滤膜过滤除菌，测定含量。在无菌条件下将所得溶液装入西林瓶中，放入温度为-30℃的冻干箱内进行预冻，再将冻干箱内真空度升至 13.33Pa 以下关闭冷冻机，通过搁置板下的加热系统缓缓加温，供给样品在升华干燥过程中所需的热量，使冻结样品的温度逐渐升高至约-20℃，药液中的水分就可升华，持续升华干燥 16 小时，直至水分除尽，当升华干燥完成后，为尽可能除去残余水分，需要进一步干燥，再干燥温度控制在 20℃。板温控制在 30℃以下，直至样品温度与板温相同，即达干燥终点。冷凝器温度达到－33℃，再干燥 12 小时，轧盖，出箱，包装即得本发明的盐酸伐昔洛韦冻干制剂。

实施例 5:

取盐酸伐昔洛韦 100mg 及右旋糖苷 50mg，加入到 1ML 注射用水中，搅拌使其溶解，按照 1.0%（w/v）的用量加入活性炭，25℃搅拌

40 分钟，脱炭过滤，加入氨水调溶液的 pH 值至 7.0，所得滤液用 0.22μm 微孔滤膜过滤除菌，测定含量。在无菌条件下将所得溶液装入西林瓶中，放入温度为-40℃的冻干箱内进行预冻，再将冻干箱内真空度升至 13.33Pa 以下关闭冷冻机，通过搁置板下的加热系统缓缓加温，供给样品在升华干燥过程中所需的热量，使冻结样品的温度逐渐升高至约-20℃，药液中的水分就可升华，持续升华干燥 15 小时，直至水分除尽，当升华干燥完成后，为尽可能除去残余水分，需要进一步干燥，再干燥温度控制在 30℃。板温控制在 30℃以下，直至样品温度与板温相同，即达干燥终点。冷凝器温度达到－33℃，再干燥 8 小时，轧盖，出箱，包装即得本发明的盐酸伐昔洛韦冻干制剂。

实施例 6:

取盐酸伐昔洛韦 100mg 及氯化钠 75mg，加入到 2.4ML 注射用水中，搅拌使其溶解，按照 1.5%（w/v）的用量加入活性炭，30℃搅拌 40 分钟，脱炭过滤，加入磷酸氢二钠调溶液的 pH 值至 6.5，所得滤液用 0.22μm 微孔滤膜过滤除菌，测定含量。在无菌条件下将所得溶液装入西林瓶中，放入温度为-50℃的冻干箱内进行预冻，再将冻干箱内真空度升至 13.33Pa 以下关闭冷冻机，通过搁置板下的加热系统缓缓加温，供给样品在升华干燥过程中所需的热量，使冻结样品的温度逐渐升高至约-20℃，药液中的水分就可升华，持续升华干燥 20 小时，直至水分除尽，当升华干燥完成后，为尽可能除去残余水分，需要进一步干燥，再干燥温度控制在 35℃。板温控制在 30℃以下，直至样品温度与板温相同，即达干燥终点。冷凝器温度达到－33℃，再干燥 10 小时，轧盖，出箱，包装即得本发明的盐酸伐昔洛韦冻干制剂。

实施例 7:

取盐酸伐昔洛韦 150mg 及甘露醇 50mg、右旋糖苷 50mg，加入到 3ML 注射用水中，搅拌使其溶解，按照 0.5%（w/v）的用量加入活性炭，20℃搅拌 40 分钟，脱炭过滤，加入磷酸氢二钾调溶液的 pH 值至 5.5，所得滤液用 0.22μm 微孔滤膜过滤除菌，测定含量。在无菌条件

下将所得溶液装入西林瓶中，置冷冻干燥机中冷冻干燥 30 小时后，轧盖，出箱，包装即得本发明的盐酸伐昔洛韦冻干制剂。

实验例 1:

注射与口服盐酸伐昔洛韦对HSV - 2 所致小鼠阴道炎的治疗作用:

体重为18 —20 克健康昆明种小鼠60只,分三组实验,每组20 只。用10TCID50HSV - 2液感染小鼠阴道,每只给0. 03ml 病毒稀释液,感染后两小时,第一组口服盐酸伐昔洛韦25mg/ kg，第二组静脉注射盐酸伐昔洛韦25mg/ kg，第三组为阴性对照组给以等量生理盐水,给药6 天,每天2 次。给药后连续观察15天。每天记录小鼠感染HSV - 2后死亡数和死亡时间,计算死亡保护率和延长生命率,公式如下: ①死亡保护率=（对照组死亡率- 实验组死亡率），②延长生命率=（实验组平均生活日数- 对照组平均生活日数）÷对照组平均生活日数×100 %。

结果见表1

表1 注射与口服盐酸伐昔洛韦治疗结果的比较

试验组	死亡率（%）	死亡保护率（%）	存活天数（天）	延长生命率（%）
1	35	55	13.1 ± 1.9	81.9
2	40	50	12.3 ± 2.3	70.8
3	90	-	7.2 ± 2.0	-

结果表明注射用伐昔洛韦与口服伐昔洛韦对HSV - 2 所致小鼠阴道炎的治疗作用无明显差别，均明显优于对照组。

虽然，上文中已经用一般性说明及具体实施方案对本发明作了详尽的描述，但在本发明基础上，可以对之作一些修改或改进，这对本领域技术人员而言是显而易见的。因此，在不偏离本发明精神的基础上所做的这些修改或改进，均属于本发明要求保护的范围。

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A VALACYCLOVIR HYDROCHLORIDE FREEZE DRIED PRODUCT AND THE PREPARATION METHOD THEREOF

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PATENT APPLICATION CLAIMS /2*

1. A type of valacyclovir hydrochloride freeze dried product, wherein it is characterized by said freeze dried product comprising being formed by thoroughly mixing the active ingredient valacyclovir hydrochloride with medically accepted excipients; the weight use ratio of the valacyclovir hydrochloride and the aforementioned excipient is 75 - 500 : 0 - 500.

2. A valacyclovir hydrochloride freeze dried product as described in Claim 1, wherein the weight use ratio of the valacyclovir hydrochloride and the aforementioned excipient is 80 - 500 : 10 - 500.

3. A valacyclovir hydrochloride freeze dried product as described in Claim 2, wherein the weight use ratio of the valacyclovir hydrochloride and the aforementioned excipient is 90 - 500 : 20 - 500.

4. A valacyclovir hydrochloride freeze dried product as described in Claim 3, wherein the weight use ratio of the valacyclovir hydrochloride and the aforementioned excipient is 100 - 500 : 25 - 500.

5. A valacyclovir hydrochloride freeze dried product as described in Claim 1, wherein the aforementioned medically accepted excipients can be selected from the one type or multiple types of the following substances: mannitol, sorbitol, sodium chloride, dextrose, sucrose, lactose, dextran.

6. Any of the valacyclovir hydrochloride freeze dried products as described in Claims 1 - 5, wherein the aforementioned freeze dried product also includes a stabilizer, analgesic, and/or buffering agent.

7. A valacyclovir hydrochloride freeze dried product as described

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* Numbers in the margin indicate pagination in the foreign text.

in Claim 1, wherein it is characterized by said method comprises the following steps: measure 75 - 500 parts (weight) valacyclovir hydrochloride, dissolve 0 - 500 parts (weight) medically accepted excipient into injection water, use a pH modulator to adjust the pH value of said solution to 5.0 - 8.5, and then freeze dry said solution.

8. A preparation method as described in Claim 7, wherein the aforementioned medically accepted excipients can be selected from the one type or multiple types of the following substances: mannitol, sorbitol, sodium chloride, dextrose, sucrose, lactose, dextran.

9. A preparation method as described in Claim 7, wherein the aforementioned pH modulator can be selected from the following eight types to make a buffer solution: sodium hydroxide, potassium hydroxide, ammonia water, phosphoric acid, citric acid, acetic acid, lactic acid, and its salt.

10. Any one of the preparation methods as described in Claims 7 - 9, wherein the aforementioned buffers also contain a stabilizer, analgesic, and/or buffering agent.

SPECIFICATION /3

A VALACYCLOVIR HYDROCHLORIDE FREEZE DRIED PRODUCT AND THE PREPARATION METHOD THEREOF

Field of the Technology

The present invention involves a type of freeze dried product and the preparation method thereof. Specifically, the present invention involves a type of valacyclovir hydrochloride freeze dried product and the preparation method thereof.

Background of the Technology

Valacyclovir hydrochloride is an acyclovir prodrug, and is a hydrochloric acid salt of the ester formed by acyclovir and valine. The molecular formula is: $C_{13}H_{20}N_6O_4 \cdot HCL$. After valacyclovir hydrochloride is orally administered, it rapidly transforms into acyclovir. In cells infected with a virus, the activity of the deoxidized tymidine kinase is further phosphorylated into three phosphates. By inhibiting DNA polymerase, and under the effect of DNA polymerase, it combines with the lengthened DNA, terminating the duplication of the illness and therefore achieving a resistance to the illness. Clinical uses for this include the treatment of shingles, genital herpes, hepatitis B, condyloma acuminate, etc. And, it is widely used in the treatment of other infections forms of herpes. Valacyclovir was first sold in England and Ireland in January 1995 and was approved by the US FDA that June. It is currently in clinical use in several dozen countries. China's Ministry of Health officially approved it in 1996 (Qinghai Medical Journal, Issue 4, Volume 31, 2001) as a new form of disease resistant nucleoside drug.

There are currently few reports regarding the preparation of valacyclovir hydrochloric acid, and, in China, there have yet to be seen any key patent documents reported. And, there is only orally administered types available inside and outside of China; There has yet to be any reports related to injectable types. The invention of an injectable type is a necessity in order to improve treatment effectiveness and to provide doctors with more options for this drug, especially for the prevention of acute diseases and to provide addition treatment options. Because the

freeze dried product is stable, convenient to use, and easy to transport and store, therefore, the preparation method for a valacyclovir hydrochloride freeze dried product was invented.

Content of the Invention

The orally administrated valacyclovir hydrochloride takes a long time for its effectiveness to commence and it is an irritant to the gastrointestinal tract if used long-term. The present invention provides a type of valacyclovir hydrochloride freeze dried product and its preparation method.

In order to overcome the aforementioned drawbacks, the present invention utilizes the following technical solution:

The present invention provides a type of valacyclovir hydrochloride freeze dried product, of which it is characterized by said freeze dried product comprising being formed by thoroughly mixing the active ingredient valacyclovir hydrochloride with medically accepted excipients. The weight use ratio of the valacyclovir hydrochloride and the aforementioned excipient is 75 - 500 : 0 - 500. Of which, the weight use ratio of the valacyclovir hydrochloride and the aforementioned excipient is optimal at 80 - 500 : 10 - 500; is even more optimal at 90 - 500 : 20 - 500; and is most optimal at 100 - 500 : 25 - 500. /4

Of which, the aforementioned medically accepted excipients can be selected from the one type or multiple types of the following substances: mannitol, sorbitol, sodium chloride, dextrose, sucrose, lactose, dextran.

In addition, the aforementioned freeze dried product also includes a stabilizer, analgesic, and/or buffering agent.

In addition, the present invention also provides a preparation method for a type of valacyclovir hydrochloride freeze dried product, of which it is characterized by said method comprises the following steps: measure 75 - 500 parts (weight) valacyclovir hydrochloride, dissolve 0 - 500 parts (weight) medically accepted excipient into injection water. Add 0.1% - 1.0% (w/v) activated carbon for injection. Heat the solution and stir for 20 - 30 minutes. Remove from heat to cool to room temperature. Filter to remove the carbon. Adjust the pH value to 5.0 - 8.5. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. Place the solution on sterile strips and pack in a tube vial. Freeze dry.

Of which, the aforementioned medically accepted excipients can be selected from the one type or multiple types of the following substances: mannitol, sorbitol, sodium chloride, dextrose, sucrose, lactose, dextran. Of which, the aforementioned pH modulator can be selected from the following eight types to make a buffer solution: sodium hydroxide, potassium hydroxide, ammonia water, phosphoric acid, citric acid, acetic acid, lactic acid, and its salt. Of which, the aforementioned phosphates include potassium phosphate, sodium phosphate, disodium phosphate, sodium dihydrogen phosphate, potassium phosphate monobasic, etc.

To further explain the invention, the aforementioned buffers also contain a stabilizer, analgesic, and/or buffering agent, such as sulfite, ascorbic acid, thiourea, cysteine, ascorbyl palmitate, vitamin E, calcium disodium edentate, calsium disodium chelate, procaine hydrochloride, lidocaine hydrochloride, docaine, sodium hydroxide, potassium hydroxide, ammonia water, phosphate, citrate, acetate, and lactate. Of which, the

aforementioned phosphates include potassium phosphate, sodium phosphate, disodium phosphate, sodium dihydrogen phosphate, potassium phosphate monobasic, etc.

For further description, the preparation method provide by the present invention comprises the following steps:

(1) Add the excipient into injection water and stir to dissolve.

(2) Add 0.1% - 1.0% (w/v) activated carbon for injection to the solution provided in the first step. Dissolve in water and stir 20 - 30 minutes. Place and let cool to room temperature. Filter out the charcoal. Adjust the pH value to 5.0 - 8.5. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. /5

(3) Under sterile conditions, pour the solution into a vial.

(4) Prepare the temperature in each of the freeze drying containers to -40°C. Place the samples in the freeze drying containers. Wait for the temperature to cool to -35°C and then freeze dry for two hours. Then, vacuum pump and gradually raise the temperature to room temperature in 15 - 30 hours. At 20°C - 30°C, freeze dry again for two hours.

For clinical use, add and dissolve in an injection use solvent (an injection use normal saline or dextrose injection solution), shake evenly and use.

The reproducibility of using the valacyclovir hydrochloride freeze dried product preparation method as the provided by the present invention is quite good (making three batches in a row will result in meeting quality requirements). The finished product rate is high (the finished product rate of three samples is greater than 98.0%). The freeze dried product

obtained exhibits an excellent appearance and can be easily re-dissolved and is well-suited for storage. It can stably be prepared for injection with dextrose and sodium chloride and the solution shows excellent clarity. The quality of the product is stable and it is easy to use. It can be used for muscle injection and also intravenous injection. It also helps prevent the issues associated with oral administration method, increasing the number of options for this type of product. It is easily used in clinical environments and increases the biological use, specifically as a better option for providing medicine for acute sufferers.

Preferred Embodiments

Preferred Embodiment 1:

Add 100 mg valacyclovir hydrochloride and 50 mg mannitol into 1 ML of injection use water. Stir to dissolve. Add activated charcoal in a 0.5% (w/v) weight use. Stir for 40 minutes at 20°C. Filter to remove the charcoal. Add in sodium hydroxide to adjust the pH value of the solution to 8.5. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. Under sterile conditions, pour the obtained solution into a vial. Place in a freeze dry system and freeze dry for 20 hours. Cap and remove. Pack to obtain the valacyclovir hydrochloride freeze dried product of the present invention.

In order to check the suitability and stability of the formulation technique of the freeze dried production of the present invention, check the blaze (4500 ± 500Lx), temperature (60°C), humidity (the corresponding humidity is 90 ± 5%) of the aforementioned freeze dried product. Of which, the results are as shown in the following table:

Affecting Factor	Settling Time (Days)	Hue	Related Quality (%)	Clarity	Content (%)
Blaze	0	White Scattered Particles	Pass	Pass	99.3
	5	White Scattered Particles	Pass	Pass	99.3
	10	White Scattered Particles	Pass	Pass	98.9
Temperature	0	White Scattered Particles	Pass	Pass	101.0
	5	White Scattered Particles	Pass	Pass	98.9
	10	White Scattered Particles	Pass	Pass	99.2
High-humidity	0	White Scattered Particles	Pass	Pass	99.5
	5	White Scattered Particles	Pass	Pass	100.2
	10	White Scattered Particles	Pass	Pass	98.8

The results in the aforementioned table show that using the valacyclovir hydrochloride freeze dried product of the present invention will produce a product that is stable towards blazing, temperature, and high humidity. Thus, there are no special requirements for packaging and storage.

Preferred Embodiment 2:

Add 200 mg valacyclovir hydrochloride and 75 mg sorbic alcohol into 2 ML of injection use water. Stir to dissolve. Add activated charcoal in a 0.5% (w/v) weight use. Stir for 30 minutes at 30°C. Filter to remove the charcoal. Add in potassium hydroxide to adjust the pH value of the solution to 8.0. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. Under sterile conditions, pour the obtained solution into a vial. Pre-freeze by placing in a freeze drying container with a temperature of -20°C and then raise the vacuum degree inside the freeze drying container to under 13.33 Pa and close the freezer. Gradually warm

with a heating system under the shelf. Provide the proper amount of heat needed to sublimate the sample in the freeze dry process so that the temperature of the frozen sample can rise to approximately -20°C. The water content in the drug solution can sublimate. Let the sublimate dry for 15 hours until the water content has been as completely removed as possible. After the sublimate completely dries to the point where some water content is still present, further drying is needed. Control the drying temperature to 15°C. Then, control the shelf temperature to under 30°C until the sample temperature is the same as the shelf to reach the dry terminal point. When the condenser temperature reaches -33°C, repeat the freeze dry step for 11 hours. Cap, remove from the container, and package to obtain the valacyclovir hydrochloride freeze dried product of the present invention.

Preferred Embodiment 3:

Add 500 mg valacyclovir hydrochloride, 150 mg sodium chloride, 100 mg dextrose, 200 mg sucrose, and 50 mg lactose into 3 ML of /7 injection use water. Stir to dissolve. Add activated charcoal in a 1.0% (w/v) weight use. Stir for 20 minutes at 40°C. Filter to remove the charcoal. Add in phosphoric acid buffer solution to adjust the pH value of the solution to 5.0. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. Under sterile conditions, pour the obtained solution into a vial. Pre-freeze by placing in a freeze drying container with a temperature of -25°C and then raise the vacuum degree inside the freeze drying container to under 13.33 Pa and close the freezer. Gradually warm with a heating system under the shelf. Provide the proper amount of heat

needed to sublimate the sample in the freeze dry process so that the temperature of the frozen sample can rise to approximately -20°C. The water content in the drug solution can sublimate. Continually sublimate dry for 17 hours until the water content has been removed. After the sublimate completely dries to the point where some water content is still present, further drying is needed. Control the drying temperature to 20°C. Then, control the shelf temperature to under 30°C until the sample temperature is the same as the shelf to reach the dry terminal point. When the condenser temperature reaches -33°C, repeat the freeze dry step for 10 hours. Cap, remove from the container, and package to obtain the valacyclovir hydrochloride freeze dried product of the present invention.

Preferred Embodiment 4:

Add 100 mg valacyclovir hydrochloride into 2.5 ML of injection use water. Stir to dissolve. Add activated charcoal in a 1.0% (w/v) weight use. Stir for 20 minutes at 40°C. Filter to remove the charcoal. Add in ammonia water to adjust the pH value of the solution to 7.5. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. Under sterile conditions, pour the obtained solution into a vial. Pre-freeze by placing in a freeze drying container with a temperature of -30°C and then raise the vacuum degree inside the freeze drying container to under 13.33 Pa and close the freezer. Gradually warm with a heating system under the shelf. Provide the proper amount of heat needed to sublimate the sample in the freeze dry process so that the temperature of the frozen sample can rise to approximately -20°C. The water content in the drug solution can sublimate. Continually sublimate dry for 16 hours until the water

content has been removed. After the sublimate completely dries to the point where some water content is still present, further drying is needed. Control the drying temperature to 20°C. Then, control the shelf temperature to under 30°C until the sample temperature is the same as the shelf to reach the dry terminal point. When the condenser temperature reaches -33°C, repeat the freeze dry step for 12 hours. Cap, remove from the container, and package to obtain the valacyclovir hydrochloride freeze dried product of the present invention.

Preferred Embodiment 5:

Add 100 mg valacyclovir hydrochloride and 50 mg dextran into 1 ML of injection use water. Stir to dissolve. Add activated charcoal in a 1.0% (w/v) weight use. Stir for 40 minutes at 25°C. Filter to /8 remove the charcoal. Add in ammonia water to adjust the pH value of the solution to 7.0. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. Under sterile conditions, pour the obtained solution into a vial. Pre-freeze by placing in a freeze drying container with a temperature of -40°C and then raise the vacuum degree inside the freeze drying container to under 13.33 Pa and close the freezer. Gradually warm with a heating system under the shelf. Provide the proper amount of heat needed to sublimate the sample in the freeze dry process so that the temperature of the frozen sample can rise to approximately -20°C. The water content in the drug solution can sublimate. Continually sublimate dry for 15 hours until the water content has been removed. After the sublimate completely dries to the point where some water content is still present, further drying is needed. Control the drying temperature to 30°C.

Then, control the shelf temperature to under 30°C until the sample temperature is the same as the shelf to reach the dry terminal point. When the condenser temperature reaches -33°C, repeat the freeze dry step for 8 hours. Cap, remove from the container, and package to obtain the valacyclovir hydrochloride freeze dried product of the present invention.

Preferred Embodiment 6:

Add 100 mg valacyclovir hydrochloride and 75 mg sodium chloride into 2.4 ML of injection use water. Stir to dissolve. Add activated charcoal in a 1.5% (w/v) weight use. Stir for 40 minutes at 30°C. Filter to remove the charcoal. Add in disodium hydrogen phosphate to adjust the pH value of the solution to 6.5. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. Under sterile conditions, pour the obtained solution into a vial. Pre-freeze by placing in a freeze drying container with a temperature of -50°C and then raise the vacuum degree inside the freeze drying container to under 13.33 Pa and close the freezer. Gradually warm with a heating system under the shelf. Provide the proper amount of heat needed to sublimate the sample in the freeze dry process so that the temperature of the frozen sample can rise to approximately -20°C. The water content in the drug solution can sublimate. Continually sublimate dry for 20 hours until the water content has been removed. After the sublimate completely dries to the point where some water content is still present, further drying is needed. Control the drying temperature to 35°C. Then, control the shelf temperature to under 30°C until the sample temperature is the same as the shelf to reach the dry terminal point. When the condenser temperature reaches -33°C, repeat the freeze dry step

for 10 hours. Cap, remove from the container, and package to obtain the valacyclovir hydrochloride freeze dried product of the present invention.

Preferred Embodiment 7:

Add 150 mg valacyclovir hydrochloride, 50 mg mannitol and 50 mg dextran into 3 ML of injection use water. Stir to dissolve. Add activated charcoal in a 0.5% (w/v) weight use. Stir for 40 minutes at 20°C. Filter to remove the charcoal. Add in dipotassium hydrogen phosphate to adjust the pH value of the solution to 5.5. Use a 0.22 μm membrane filter to filter out the bacteria. Measure the content. Under sterile /9 conditions, pour the obtained solution into a vial. After placing in a freeze dryer and freeze drying for 30 hours, cap, remove from the container, and package to obtain the valacyclovir hydrochloride freeze dried product of the present invention.

Experiment Example 1:

The treatment affect of injected and orally administrated valacyclovir hydrochloride on HSV-2 that causes vaginitis in mice.

Prepare 60 Kunming mice of a body weight between 18 and 20 g. Separate into three groups (20 per group) for the experiment. Use a 10TCID50HSV-2 solution to infect the vaginas of the mice. Each mouse receives 0.03 ml of the disease thinner. Two days after infection, orally administer valacyclovir hydrochloride (25 mg/kg) to the first group. Intravenously inject 25 mg/kg valacyclovir hydrochloride into the second group. The third group is the negative control group and is given an equal amount of physiological saline. Administer accordingly twice a day for six days. After the treatment, continue to observe for 15 days. Record the number

of HSV-2 infected mice that have died each day. Also record the time of death. Calculate the death protection rate and extended life rate by using the following formula: (1) Death Protection Rate = (Control Group Death Rate - Experimental Group Death Rate), (2) Extended Life Rate = (Average Number of Days Alive of the Experimental Group - Average Number of Days Alive of the Control Group) ÷ Average Number of Days Alive of the Control Group x 100%.

See Table 1 for the results.

Table 1. Results of a Comparison Between Injection and Oral Valacyclovir Hydrochloride Treatments

Test Group	Death Rate (%)	Death Protection Rate (%)	Number of Days Alive (days)	Extended Life Rate (%)
1	35	55	13.1 ± 1.9	81.9
2	40	50	12.3 ± 2.3	70.8
3	90	-	7.2 ± 2.0	-

The results show that there is no clear difference between injected valacyclovir hydrochloride and orally administered valacyclovir hydrochloride on the treatment of vaginitis on mice caused by HSV-2. And, both forms of treatment surpass the control group.

Although the aforementioned text has provided a general description as well as specific embodiments to describe in detail the present invention, any modification or improvement that can be done on the basis of the present invention can be easily understood by those familiar with the technology. Therefore, such modifications or improvements do not deviate from the basis of the spirit of the present invention and fall under the scope of protection as set forth in the claims of the present invention.

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Amino acid ester prodrugs of acyclovir

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Summary

Eighteen amino acid esters of the antiherpetic drug, acyclovir, were synthesized as potential prodrugs for oral administration. The esters were examined for *in vitro* antiviral activity against herpes simplex virus Type 1 (HSV-1). They were found to have less potency than the parent compound. Their efficiencies as prodrugs were evaluated in rats by measuring the urinary recovery of acyclovir. Ten prodrugs produced greater amounts of the parent drug in the urine. The L-amino acid esters were better prodrugs than the corresponding D- or D,L-isomers, suggesting the involvement of a stereoselective transporter. The L-valyl ester, 256U87, was the best prodrug. Sixty three per cent of its administered dose was excreted as acyclovir in the urine, a considerable improvement over acyclovir itself, for which this value was 19%. Since 256U87 was stable in aqueous solutions, its conversion to acyclovir *in vivo* was probably enzyme catalyzed. This L-valyl ester prodrug of acyclovir is now undergoing clinical evaluation.

Introduction

Acyclovir (9-[(2-hydroxyethoxy)methyl]-9H-guanine, Zovirax®, 1) is a widely used agent for the treatment and prophylaxis of infections caused by the herpes group of viruses. The important human pathogens of this group are not equally sensitive to the drug. For example, the IC_{50} values ($\mu g\ ml^{-1}$) obtained in the extensive testing *in vitro*, showed the following ranges: against herpes simplex virus type 1 (HSV-1), 0.01–0.7; HSV-2, 0.01–3.2; varicella zoster virus (VZV), 0.3–10.8 and human cytomegalovirus (HCMV) 2.0–50 (O'Brien and Campoli-Richards, 1989). Thus, plasma concentrations of acyclovir achieved in humans after oral dosing with a single 200 mg capsule are adequate for the inhibition of HSV-1 or HSV-2. However, for the treatment of VZV infections and for the suppression of HCMV infections, multiple, high doses of oral or intravenous drug are necessary (Huff *et al.*, 1988; Meyers *et al.*, 1988; Peterslund, 1988; Wood *et al.*, 1988; Balfour Jr. *et al.*, 1989; Morton and Thomson, 1989). Suppressive therapy in immunocompromised patients with sub-optimal oral drug doses can lead to less sensitive strains of HSV and VZV (Barry *et al.*, 1985; Engel *et al.*, 1990; Hill *et al.*, 1991; Hoppenjans *et al.*, 1990; Jacobson *et al.*, 1990). Because of the limited oral bioavailability (15–21%) of acyclovir (de Miranda and Blum, 1983), plasma levels adequate for inhibition of less sensitive viruses can not be achieved easily. Consequently, in some clinical situations, it is necessary to administer the drug intravenously to achieve higher plasma levels for optimal efficacy against less sensitive viruses.

Thus, a highly efficient and safe prodrug of acyclovir, that can be given orally and that will achieve plasma levels of drug comparable to intravenous dosing has been sought by Burroughs Wellcome laboratories for more than a decade. Two congeners of acyclovir with alterations in the 6-substituent of the purine ring (Table 1, Fig. 1) have been extensively evaluated. The first, the 6-amino congener, 2-[(2,6-diamino-9H-purin 6-yl) methoxy]-ethanol, 2, is incompletely converted to acyclovir by adenosine deaminase (Good *et al.*, 1983). The second, the 6-deoxy congener, 2-[(2-amino-9H-purin-9-yl)methoxy]ethanol, desciclovir, 3, is dependent on xanthine oxidase for conversion to acyclovir (Krenitsky *et al.*, 1984). Neither compound has a chronic toxicity profile in experimental animals as favourable as that of acyclovir itself (G. Szczech, personal communication). The toxicity of the two congeners, encountered in laboratory animals, was hypothesized to be the result of phosphorylation of the unconverted prodrug. Therefore, we initiated a programme to develop an effective prodrug that could not be phosphorylated prior to conversion to acyclovir.

Many other investigators have studied prodrugs of acyclovir (Colla *et al.*, 1983; Selby *et al.*, 1984; Welch *et al.*, 1985; Kumar *et al.*, 1988; Bundgaard *et al.*, 1989, 1991; Štimac and Kobe, 1990). The work of Colla and colleagues is particularly relevant to this paper. Based on the analysis of a small group of amino acid esters (as hydrochloride salts), mostly the simple derivatives (glycyl, 4, α-L-alanyl, 5, β-alanyl, 6), they proposed that such water-soluble amino acid esters of acyclovir are suitable prodrugs for ophthalmic and intramuscular administration. *In vitro* experiments measuring the effects of

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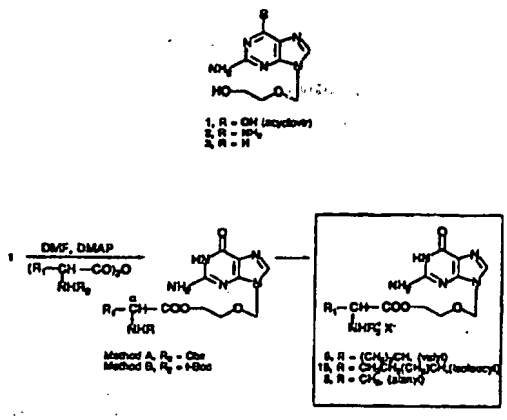

Fig. 1. Chemical synthesis of amino acid ester prodrugs of acyclovir.

these compounds on the replication of HSV-1 and HSV-2 in primary rabbit kidney cell cultures showed that the esters were somewhat less potent than the parent drug; their IC_{50}'s values ranged from 0.1 to 0.8 μg ml^{-1}, compared to 0.08 μg ml^{-1} for acyclovir. This level of antiviral activity suggested that the esters were hydrolyzed to the parent compound. *In vivo*, the glycyl ester, 4, as a 1% borate eyedrop solution (pH 5.7), demonstrated efficacy against HSV-1 keratitis in the rabbit. However, solutions of 4 at pH 7.4 and physiological temperature were 50% hydrolyzed after 4 h, and completely hydrolyzed after 1 d. Instability has been a common shortcoming of amino acid esters (Kovach *et al.*, 1981; Cho and Haynes, 1985; Johnson *et al.*, 1985).

In this study, we have determined that some amino acid esters of acyclovir with more complex side chains than glycyl and alanyl are good prodrugs for oral use. The gastrointestinal absorption of these esters is greater than that of the parent drug or the simpler amino acid esters. Moreover, the complex amino acid esters have better stability in aqueous solutions.

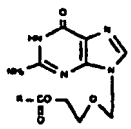

Table 1. Chemical data and oral bioavailability of amino acid prodrugs of acyclovir.

Compound No.	Ester	R	m.p. °C	% Yield	Method	Recrystallization solvent	Urinary recovery of acyclovir (% dose)
4	glycyl (2-amino-acetate)	CH_2NH_2	142–180	74	A	H_2O	30
11	D,L-alanyl	$CH_3CH(NH_2)$	179	57	A	H_2O-EtOH	31
12	D-alanyl	$CH_3CH(NH_2)$	140–164	81	A	H_2O-IPrOH	14
5	L-alanyl	$CH_3CH(NH_2)$	142–165	76	A	H_2O-IPrOH	42
8	N-methyl L-alanyl	$CH_3CH(NHCH_3)$	*	100	B	not recry	6
6	B-alanyl (3-amino-propionate)	$(CH_2)_2(NH_2)$	145–195	24	A	H_2O-EtOH	17
13	L-2-aminobutyrate	$CH_3CH_2CH(NH_2)$	*	100	B	iPrOH-EtOH	50
14	D,L-valyl	$(CH_3)_2CHCH(NH_2)$	124–126	24	A	H_2O-EtOH	30
15	D-valyl	$(CH_3)_2CHCH(NH_2)$	185–188	53	A	not recry	7
9	L-valyl	$(CH_3)_2CHCH(NH_2)$	150–195	60	A	H_2O-EtOH	53
16	D,L-norvalyl	$CH_3CH_2CH_2CH(NH_2)$	148–148	84	A	H_2O-EtOH	18
17	D,L-isoleucyl	$CH_3CH_2CH(CH_3)CH(NH_2)$	145–148	81	A	H_2O-IPrOH	25
18	L-isoleucyl	$CH_3CH_2CH(CH_3)CH(NH_2)$	180–182	92	A	not recry	43
19	L-leucyl	$(CH_3)_2CHCH_2CH(NH_2)$	140–147	57	A	MeOH-EtOH	19
7	L-methionyl	$CH_3SCH_2CH_2CH(NH_2)$	125–138	21	A†	H_2O-IPrOH	28
20	D,L-lysyl	$NH_2CH_2CH_2CH_2CH_2CH(NH_2)$	142–154	70	A	H_2O-IPrOH-acetone	18
21	L-prolyl	o-$(CH_2)_4N$	170–185	16	A	H_2O-IPrOH	21
22	D,L-phenylalanyl	$C_6H_5CH_2CH_2(NH_2)$	212–219	57	A	MeOH-acetone-THF	17
1	acyclovir, po						19
1	acyclovir, iv						86
3	(2-[(2-Amino-6H-purin-9-yl)methoxy) ethanol‡						85
2	2-(2,6-Diamine-9H-purin-9-yl)methoxy) ethanol						26

*Freeze-dried; too hygroscopic to measure mp.
†The reaction mixture had to be recharged twice with large amounts of palladium catalyst (1:1 w/w).
‡Desiclovir.

Table 2. ^{1}H-NMR chemical shifts (Ppm downfield from TMS in DMSO-d_6).

	L-valine-CBZ (10)	L-valine (9)	L-alanine (5)	L-methionine (7)	L-proline (21)	L-isoleucine (18)	Glycine (4)
NH	10.64 (s)	10.90 (s)	10.79 (s)	10.85 (s)	11.00 (s)	10.87 (s)	10.77 (s)
2-NH_2	6.51 (s)	6.76 (s)	6.65 (s)	6.73 (s)	6.80 (s)	6.70 (s)	6.64 (s)
H-8	7.81 (s)	7.83 (s)	7.81 (s)	7.82 (s)	8.12 (s)	7.97 (s)	7.82 (s)
N-CH_2-O	5.35 (s)	5.33 (s)	5.54 (s)	5.35 (s)	5.38 (s)	5.38 (s)	5.36 (s)
NH_3+		8.54 (brs)	8.46 (brs)	8.62 (brs)	8.97 (brs)	8.48 (brs)	8.34 (brs)
$CH_2OC(O)$	4.22 (m)	4.33 (m)	4.32 (m)	4.32 (m)	4.32 (m)	4.28 (m)	4.23 (m)
CH_2O	3.67 (m)	3.71 (m)	3.70 (m)	3.70 (m)	3.72 (t)	3.73 (m)	3.69
αCH	3.90 (m)	3.82 (m)	4.03 (q)	4.08 (t)	4.08 (m)	3.87 (m)	–
$CH(CH_3)_2$	1.93 (m)	2.11 (m)	–	–	–	–	–
$(CH_3)_2$	0.81 (d)	0.69 (d)	–	–	–	0.81 (m)	–
CH_3	–	–	1.32 (d)	–	–	–	–
SCH_3	–	–	–	1.99 (s)	–	–	–
CCH_2	–	–	–	2.48 (m)	–	–	–
CH_2S	–	–	–	2.48 (m)	–	–	–
CH_2N	–	–	–	–	3.72 (m)	–	–
CH_2 (ring)	–	–	–	–	3.16 (m)	–	–
CH_2 (ring)	–	–	–	–	1.85 (m)	–	–
β-CH	–	–	–	–	–	1.62 (m)	–
CH_2CH_3	–	–	–	–	–	1.25 (m)	–
α CH_2	–	–	–	–	–	–	3.76 (m)
NHCO	7.65 (d)	–	–	–	–	–	–
ArH	7.35	–	–	–	–	–	–
CH_2Ar	5.03 (s)	–	–	–	–	–	–

Results

Chemistry

The amino acid esters were prepared in two steps (Fig. 1), using a slightly modified method of Colla (Colla *et al.*, 1983). In a majority of the syntheses, the carbobenzyloxy group (N-Cbz) was used to protect the amine function of the amino acid (method A, see Materials and Experimental procedures). A solution of acyclovir in dimethylformamide (DMF) was treated with the N-protected amino acid using the coupling agent dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino) pyridine (DMAP) as a catalyst, to produce the N-Cbz blocked ester derivative. Deprotection by catalytic hydrogenation in the presence of HCl gave the target aminoacyl esters as the hydrochloride salts (method A). For the synthesis of the N-methyl L-alanyl congener, **8**, the t-butylcarbobenzoxyl (t-Boc) blocking group was used, and deprotection was effected with trifluoroacetic acid (method B). In almost all cases, the target salts analysed for water, and some contained excess HCl or other solvents. The presence of these components, indicated by the elemental analyses was confirmed by NMR data. The ^{1}H and ^{13}C NMR characteristics of all esters are listed in Tables 2 and 3.

A broad variety of amino acid esters was synthesized to ascertain the effect of different types of substituents adjacent to the carbonyl function. We investigated the occurrence of racemization only in the synthesis of the L-valyl ester, **9**. Most of the batches of the N-Cbz-valyl intermediate, **10**, produced by coupling pure N-Cbz-L-valine with acyclovir, were determined to have 2–3% of the D-isomer. The enantiomeric ratio was estimated by HPLC on a chiral column (Chiracel OD, Diacel, Chiracel Technologies Inc., Exton, PA, USA) with EtOH and MeOH (65:35) containing 0.1% trifluoroacetic acid as the mobile phase. We suspect that this slight racemization was caused by the strongly basic catalyst, DMAP. No additional racemization occurred in the reduction step, as determined by analysis of the batches of **9** on a Crown Pak CR(+) (Diacel) column with a mobile phase of aqueous perchloric acid, pH 2.

Bioavailability

The bioavailability of acyclovir, after dosing rats by gavage (25 mg kg^{-1}) with the various amino acid ester prodrugs, was estimated by determining the total amount of acyclovir recovered in the urine over a 40-h period. Acyclovir and the prodrugs were assayed by HPLC (see Materials and Experimental procedures). The esters themselves were not detected in the urine, indicating their extensive *in vivo* hydrolysis to acyclovir. The bioavailability data for the esters, expressed as per cent of prodrug (on a molar basis) excreted in the urine as acyclovir, are summarized in Table 1. The validity of estimating acyclovir bioavailability in the rat from its urinary excretion is supported by observations that acyclovir is virtually unmetabolized, and less than 1%

Table 3. ^{13}C-NMR chemical shifts (Ppm in DMSO-d_6).

Carbons	L-valine (9)	L-alanine (5)	L-proline (21)	L-valine-CBZ (10)
C=O	168.71	169.91	168.73	171.65
C-6	156.63	156.62	156.00	156.70
C-2	154.09	154.08	154.20	153.82
C-4	151.08	151.30	151.00	151.34
C-8	137.55	137.55	137.56	137.55
C-5	116.84	116.33	114.81	116.46
NCH_2O	71.68	71.74	72.08	71.71
CH_2O	66.28	66.11	66.21	66.45
CH_2OCO	64.13	64.29	64.49	65.47
αC	57.09	47.66	58.31	59.58
CH	29.23	–	–	25.57
CH_3(2)	18.13, 17.24	–	–	18.73, 17.89
CH_3	–	15.51		–
CH_2N	–	–	45.23	–
CH_2 (ring)	–	–	27.71	–
CH_2 (ring)	–	–	22.89	–
C=O (Cbz)	–	–	–	156.28
Arom	–	–	–	136.82
Arom	–	–	–	128.25
Arom	–	–	–	127.74
Arom	–	–	–	127.66
CH_2Ph	–	–	–	63.04

of the dose is secreted into the bile (de Miranda *et al.*, 1981).

Clearly, the L-valyl ester, 256U87, **9**, provided the best acyclovir bioavailability (63%), followed by the L-2-aminobutyrate, **13** (50%), the L-isoleucyl, **18** (43%), and the L-alanyl, **5** (42%) esters. In contrast, after oral administration of acyclovir to rats ($n = 4$), the urinary recovery was only 19 ± 8% of the dose (de Miranda *et al.*, 1981). An N-protected amino acid ester (the D, L-N-Cbz-alanyl, **23**) was evaluated but showed low bioavailability (8%). In the cases where the D- and L-isomers were compared (Table 1), the L-isomer was more efficient as a prodrug. For comparison, two other extensively studied acyclovir oral prodrugs, the 2,6-diaminopurine derivative, **2**, and the 2-aminopurine analogue, **3**, gave values of 26% and 65%, respectively, for the acyclovir urinary recovery in rats.

In pharmacokinetic studies with 256U87, **9**, in rats ($n = 3$), after a dose of 35 mg kg^{-1} by gavage, prodrug cleared from the plasma rapidly and was undetectable by 1 h post dose. Acyclovir mean peak plasma concentration (12.7 μM) and area under the curve (17.3 μM h^{-1}) were 7.8 and 3.6-fold higher, respectively, than after an equivalent dose of acyclovir. The plasma half-life ($t_{1/2}$) of acyclovir was approximately the same (1 h) after dosing with either the prodrug or acyclovir itself.

Aqueous solubility

All the amino acid esters of acyclovir were soluble in water at room temperature, a striking contrast to the poor solubility of the parent drug. The solubility of **9** was 174 mg ml^{-1}, a dramatic increase over that of acyclovir (1.3 mg ml^{-1}).

Aqueous stability

The aqueous stability of the glycyl, **4**, L-alanyl, **5**, L-valyl, **9**, and L-isoleucyl, **18**, derivatives was examined at 37°C at pH 6 (sodium phosphate buffer), pH 7.4 (sodium phosphate buffered saline, (PBS), and pH 8 (sodium phosphate buffer). Hydrolysis to acyclovir was detected by HPLC using a C18 reversed-phase column. The calculated $t_{1/2}$s are shown in Table 4. The glycyl and L-alanyl esters were considerably less stable than the L-valyl and L-isoleucyl analogues; the order of decreasing stability was **18** > **9** >> **4** > **5**. The stability of all four esters decreased with increasing pH.

Antiviral activity

The esters were examined for antiviral activity *in vitro* against HSV-1. Results are shown in Table 5. The amino acid esters were active against HSV-1 replication with IC_{50}

Table 4. Half-lives (h) of amino acid ester prodrugs of acyclovir at 37°C.

Ester	Compound no.	pH 6	pH 7.4	pH 8
L-Valyl	9	95	13	9.5
L-Isoleucyl	18	160	ND*	14
L-Alanyl	5	11	ND	1.2
Glycyl	4	16	ND	2.1

ND*, not determined.

Table 5. *In vitro* testing of amino acid esters against HSV-1*.

Compound no.	Ester	IC_{50} (μM)
15	D-valyl	2.3
9	L-valyl (256U87)†	0.84
18	L-isoleucyl	<8.25
19	L-leucyl	1.25
6	3-aminopropionate	10.5
4	glycyl (2-aminoacetate)	1.7
7	L-methionyl	1.4
12	D-alanyl	1.2
5	L-alanyl	2.8
20	D,l-lysinyl	1.42
15	L-prolyl	1.08
22	D,l-phenylalanyl	0.93
13	2-aminobutyrate	<10
1	acyclovir	0.1

*SC 18 strain, Vero cells.
†$CCID_{50}$ (Vero cells) = >500 μM.

values ranging from 0.84 μM (L-valyl 256U87, 9) to 10.5 μM (3-aminopropionate, 6). The value for acyclovir was 0.1 μM. Antiviral activity of the esters was probably the result of acyclovir generated from partial hydrolysis of the esters in the test system (Vero cells). No cytotoxicity to Vero cells was detected with any of the compounds up to concentrations of 100 μM. The concentrations of 256U87, 9, required to inhibit the growth of 50% of Vero cells was greater than 500 μM.

Toxicological testing

The metabolic products of 256U87, 9, in rats were acyclovir, which has a well known safety record in clinical use, and an essential amino acid, L-valine. Within 90 min of dosing, there was no detectable prodrug in plasma. Not surprisingly, therefore, when adjusted for equivalent plasma levels, 256U87, 9, had the same safety profile as acyclovir in a variety of subchronic and chronic tests in several species (G. Szczech, personal communication).

Discussion

Compared to acyclovir (19% urinary recovery), ten prodrugs produced greater amounts of the parent drug resulting in higher urinary recoveries after oral administration. Water solubility was not directly related to oral absorption, since all the ester prodrugs in Table 1 were soluble but provided varied bioavailabilities of acyclovir. On the other hand, the stereochemistry of the amino acid in the prodrug esters had a marked effect on absorption. In the cases where the D-isomer was directly compared with the L-isomer, there was a decided preference for the L-isomer. The racemates were intermediate in efficiency of absorption. The preference for the L- vs. D-isomer and for the naturally occurring branched chain amino acids, L-valyl and L-isoleucyl, suggests that a stereospecific transporter may contribute to the improved absorption of these esters.

The structure of the side chain of the amino acid esters of acyclovir had a profound effect on the efficiency of prodrug absorption. With the simple aliphatic straight side chains, the optimal length for the chain adjacent to the alpha carbon (see Table 1) was two carbons (5 vs. 4; 13 vs. 16). A methyl group substitution on the beta carbon of the amino acid further enhanced prodrug efficiency (9 vs. 13; 18 vs. 16), but an ethyl side chain slightly decreased it (18 vs. 9). Of the prodrug compounds studied, the L-valyl derivative, 256U87, 9, had the optimal combination of chain length and branching at the beta carbon.

The chemical stability of the most efficient ester, 256U87, 9, contrasted with its extensive *in vivo* hydrolysis to acyclovir. The rapid conversion of the prodrug to acyclovir *in vivo*, and the virtual absence of the unchanged prodrug in the urine suggests the involvement of a very efficient enzymatic process. The conversion is probably a result of 'first pass' metabolism. Further studies that confirm this hypothesis will be published elsewhere (P. de Miranda and T. Burnette)

The antiherpes testing of the amino acid esters *in vitro* showed a range of IC_{50} values but all were higher than acyclovir itself (Table 5). Since antiviral action is dependent on phosphorylation of the parent compound (a fact which has been exhaustively documented), a priori, the intact esters, having no free hydroxyl group capable of phosphorylation, would not be expected to exhibit any antiviral action.

The potential clinical benefits of a highly bioavailable, safe, oral prodrug of acyclovir are outlined in the introduction section of this paper. However, the subject is worthy of further discussion. In the case of acyclovir resistant HSV infections, high dose intravenous therapy is usually effective (Engel *et al.*, 1990), demonstrating the utility of reaching high plasma levels. Consequently, 256U87 should provide a more convenient treatment for such infections. Most of the resistance to acyclovir is encountered in immunocompromised patients where inadequate exposure to drug has occurred. The use of 256U87 should ensure that optimal exposure is achieved and thereby decrease the frequency of resistance. In VZV infections, the use of 256U87 should obviate the need for multiple, high dose administration of acyclovir and also decrease the frequency of resistance. Currently, the leading drug for the treatment of HCMV infections is ganciclovir. Its toxicity, very poor oral bioavailability, and frequent incompatibility with zidovudine create an urgent need for better drug treatment. Although acyclovir is less potent than ganciclovir against HCMV, the ability to achieve high plasma levels of acyclovir by oral administration of 256U87 represents a clinical opportunity especi-

ally for suppression in immunocompromised patients, where oral administration is of particular importance.

In summary, 256U87, **9**, the L-valyl ester of acyclovir, as a hydrochloride salt, is a prodrug that, given orally, can produce higher plasma levels and three- to four-fold higher bioavailability of acyclovir, than administration of the parent compound itself. The prodrug was stable in aqueous acidic solutions. *In vivo* it was rapidly absorbed and metabolized to the parent. Thus far in toxicological studies, 256U87 has the same safety profile as acyclovir. It is currently being evaluated in clinical trials for more effective oral treatment and suppression of the herpes group infections.

Materials and Experimental procedures

Chemical procedures

Melting points were determined with a Thomas Hoover melting point apparatus (Thomas Scientific Co., Swedesboro, NJ, USA) and are uncorrected. Ultraviolet spectra were recorded with a Varian DMS-100 spectrophotometer or a Beckman DU-70 spectrophotometer. NMR spectra were recorded using a Varian XL-200 or a Varian XL-300 spectrometer. Elemental microanalyses were determined by Atlantic Microlabs, Atlanta, GA, USA. Preparative column chromatography was done by the flash chromatography technique on Silica Gel (40–63 μM, E. Merck No. 9385). Solvents were removed by rotary evaporation (Buchler flash evaporator) in a temperature-controlled water bath.

Method A: 2-[(2-Amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl L-valinate hydrochloride, 256U87, 9. Acyclovir (2.0 g, 8.88 mM) was dissolved in dry DMF (150 ml) by warming on a steam bath. Successively, DMAP (0.154 g, 1.25 mM), N-Cbz-L-valine (3.01 g, 12.0 mM), and DCC (3.0 g, 14.4 mM) were added to the cooled solution. The solution was stirred under a nitrogen atmosphere at ambient temperature for 16 h. The mixture was recharged with additional DMAP (0.154 g, 1.25 mM), N-Cbz-L-valine (3.01 g, 12.0 mM), and DCC (3.0 g, 14.4 mM), and stirring was continued at ambient temperature for 2 days. The mixture was filtered, the DMF was removed from the filtrate *in vacuo*, and the residue was chromatographed on silica gel, using 1:4 MeOH CH_2Cl_2 as the eluant, to give 3.75 g (92%) of the desired intermediate, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl *N*-Cbz-L-valinate, **10**, as a white solid. The ^1H- and ^{13}C-NMR spectra were consistent with the desired structure. A solution of **10** (3.73 g, 8.14 mM) in MeOH (130 ml), THF (tetrahydrofuran, 65 ml), and H_2O (25 ml) was added to 0.5 N aqueous HCl (18 ml) and 377 mg of 5% palladium on charcoal. The mixture was shaken in a Parr apparatus under an initial pressure of 50 psi of hydrogen at ambient temperature for 18 h. The mixture was filtered, the catalyst was washed with MeOH, and the combined washings and filtrate were evaporated *in vacuo* at a bath temperature <60°C. The residue was recrystallized from H_2O/2-propanol to yield 1.762 g (60)% of **9** as the HCl salt, which analysed for 1.0 mole of H_2O ($C_{13}H_{23}ClN_6O_6$) C, H, N, Cl. The ^1H- and ^{13}C-NMR spectra were satisfactory for the desired structure. UV (H_2O): λ_{max} 252.8 mμ (ε 8530), sh 273.2 mμ (ε 5782).

Method B: 2-[(2-Amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl N-methyl-L-alanate triflate, 8. A mixture of N-*t*-Boc-N-methyl-L-alanine (1.0 g, 4.92 mM) and DCC (0.60 g, 2.91 mM) in 40 ml CH_2Cl_2 was stirred for 3 h at ambient temperature under nitrogen, and then filtered; the filtrate was evaporated *in vacuo*. The anhydride was dissolved in 50 ml dry DMF and treated with acyclovir (0.54 g, 2.40 mM) and DMAP (0.030 g, 0.35 mM). After 40 h, the solvent was removed *in vacuo*, and the impure solid was recrystallized three times from 5% CH_2Cl_2/MeOH to provide 0.76 g (77%) of the desired intermediate, **23**, as a white powder, mp 181°C (dec). A solution of **23** (0.58 g, 1.35 mM) in 25 ml of freshly distilled trifluoroacetic acid was stirred at 0°C for 30 min. The excess acid was removed *in vacuo*, and the residue was dissolved in 4 ml H_2O and freeze-dried for 18 h. The gum was redissolved in 4 ml H_2O and freeze-dried again to provide a quantitative yield of **8** as a hygroscopic, fluffy white solid ($C_{12}H_{18}N_6O_4 \times 14/5\ C_2HF_3O_2$, 1/10 H_2O) C, H, N. UV (H_2O): λ_{max} 251 mμ (ε 14230), sh 273.2 mμ (ε 9750).

^1H and ^{13}C NMR data are listed in Tables 2 and 3, respectively.

Biological procedures

Long Evans rats (2 males) were administered 25 mg kg^{-1} of prodrug in aqueous solution by gavage. The animals were housed in pairs in a single Nalgene metabolism cage (Nalge Company, Rochester, NY, USA) and given food and water *ad libitum*. Urine was collected over the 0–24 h and 24–48-h post-dose periods, and then filtered through 0.22-μm filters (Millex-GS; Millipore Corporation, Bedford MA, USA) or ultrafiltered through Centrifree micropartition units (Amicon Corporation, Danvers, MA, USA) by centrifugation at 2000 × g for 15 min. The urine filtrates were analysed by reversed-phase high-performance liquid chromatography (HPLC). An aliquot of the sample was applied to a C18 analytical column (Adsorbosphere, 5 μm, 4.6 mm ID × 25 cm; Alltech Associates/Applied Science, Avondale, PA, USA) equipped with a compatible guard column. The column was eluted at a flow rate of 1 ml min^{-1} with the following three-step gradient: (step 1) a 30-min linear gradient from 100% mobile phase A (25 mM ammonium formate buffer, pH 3.5) to 90% A and 10% mobile phase B (50% CH_3CN in 50 mM ammonium acetate buffer, pH 5.5); (step 2) a 5-min linear gradient from 90% A and 10% B to 100% B; (step 3) a 10-min isocratic elution with 100% B. The column was equilibrated in 100% A for 15 min between samples. The UV absorbance of the column effluent was monitored at 254 and 280 nm. Estimates of the oral bioavailability were expressed as the per cent of the dose (on a molar basis) that was excreted in the urine as acyclovir. The data on urinary recovery of acyclovir in Table 1 was estimated to have typical variability of ±4%. Pharmacokinetic studies in rats were performed by methods described earlier (Burnette *et al.*, 1991). Antiviral evaluation was performed using a plaque reduction assay (Collins *et al.*, 1982) using HSV-1 SC 16 strain in Vero cells.

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References

Balfour, H.H. Jr, Chace, B.A., Stapleton, J.T., Simmons, R.L., and Fryd, D.S. (1989) A randomized, placebo-controlled trial of oral acyclovir for the prevention of cytomegalovirus disease in recipients of renal allografts. *N Eng J Med* **320**: 1381–1387.

Barry, D.W., Nusinoff-Lehrman, S., and Ellis, M.N. (1985) Viral resistance, clinical experience. *Scand J Infect Dis* Suppl. **47**: 155–164.

Bundgaard, H., Falch, E., and Jensen, E. (1989) A novel solution-stable, water soluble prodrug type for drugs containing a hydroxyl or an NH-acid group. *J Med Chem* **32**: 2503–2507.

Bundgaard, H., Jensen, E., and Falch, E. (1991) Water soluble, solution stable, and bioliable N-substituted (aminomethyl) benzoate ester prodrugs of Acyclovir. *Pharm Res* **8**, 9: 1087–1093.

Burnette, T.C., Koszalka, G.W., Krenitsky, T.A., and de Miranda, P. (1991) Metabolic disposition and pharmacokinetics of the antiviral agent 6-methoxypurine arabinoside in rats and monkeys. *Antimicrob Agents Chemother* **35**: 1165–1173.

Cho, M.J. & Haynes, L.C. (1985) Serum-catalyzed hydrolysis of metronidazole amino acid esters. *J Pharm Sci* **74**: 883–885.

Cobo, L.M., Foulks, G.N., Liesegang, T., Lass, J., Sutphin, J.E., Wilhelmus, K., Jones, D.B., Chapman, S., Segreti, A.C., and King, D.H. (1986) Oral acyclovir in the treatment of acute herpes zoster ophthalmicus. *Ophthalmology* **93**: 763–770.

Colla, L., De Clercq, E., Busson, R. & Vanderhaeghe, H. (1983) Synthesis and antiviral activity of water soluble esters of acyclovir [9-[(2-hydroxyethoxy)methyl]guanine]. *J Med Chem* **26**: 602–604.

Collins, P., Appleyard, G., and Oliver, N.M. (1982) Sensitivity of herpesvirus isolates from acyclovir clinical trials. *Am J. Med* **73** (1A): 380–382.

de Miranda, P., and Blum, M.R. (1983) Pharmacokinetics of acyclovir after intravenous and oral administration. *J Antimicrob Chemother* **12** Suppl B: 29–37.

de Miranda, P., Krasny, H.C., Page, D.A., and Elion, G.B. (1981) The disposition of acyclovir in different species. *J Pharmacol Exp Ther* **219**: 309–315.

Engel, J.P., Englund, J.A., Fletcher, C.V., and Hill, E.L. (1990) Treatment of resistant herpes simplex virus and continuous-infusion acyclovir. *JAMA* **263** (12): 1662–1664.

Good, S.S., Krasny, H.C., Elion, G.B., and de Miranda, P. (1983) Disposition in the dog and the rat of 2,6-diamino-9-(2-hydroxyethoxymethyl)purine (A134U), a potential prodrug of acyclovir. *J Pharmacol Exp Ther* **227**: 644–651.

Hill, E.L., Hunter, G.A., and Ellis, M.N. (1991) In vitro and in vivo characterization of herpes simplex virus clinical isolates recovered from patients infected with human immunodeficiency virus. *Antimicrob Agents Chemother* **35**: 2322–2328.

Hoppenjans, W.B., Bibler, M.R., Orme, R.L., and Solinger, A.M. (1990) Prolonged cutaneous herpes zoster in acquired immunodeficiency syndrome. *Arch Dermatol* **126**: 1048–1050.

Huff, J.C., Bean, B., Balfour, H.H., Jr, Laskin, O.L., Connor, J.D., Corey, L., Bryson, Y.J., and McGuirt, P. (1988) Therapy of herpes zoster with oral acyclovir. *Am J Med* **85**: (Suppl 2A): 84–89.

Jacobson, M.A., Berger, T.G., Fikrig, S., Becherer, P., Mochr, J.W., Stanat, S.C., and Biron, K.K. (1990) Acyclovir-resistant varicella zoster virus infection after chronic oral acyclovir therapy in patients with the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* **112**: 187–191.

Johnson, K., Amidon, G.L., and Pogany, S. (1985) Solution kinetics of a water-soluble hydrocortisone prodrug: Hydrocortisone-21-lysinate. *J Pharm Sci* **74**: 87–89.

Kovach, I.M., Pitman, I.H., and Higuchi, T. (1981) Amino acid esters of phenols as prodrugs: Synthesis and stability of glycine, β-aspartic acid, and α-aspartic acid esters of p-acetamidophenol. *J Pharm Sci* **70**: 881–885.

Krenitsky, T.A., Hall, W.W., de Miranda, P., Beauchamp, L.M., Schaeffer, H.J., and Whiteman, P.D. (1984) 6-Deoxyacyclovir: A xanthine oxidase-activated prodrug of acyclovir. *Proc Natl Acad Sci USA* **81**: 3209–3213.

Kumar, S., Oakes, F.T., Wilson, S.R., and Leonard, N.J. (1988) Synthesis and structure of a fluorescent, tricyclic analogue of 2′-deoxyadenosine and of a prodrug by N-annelation of 2′-deoxyguanosine and 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir), respectively. *Heterocycles* **27**: 2891–2901.

Meyers, J.D., Reed, E.C., Shepp, D.H., Thornquist, M., Dandliker, P.S., Vicary, C.A., Flournoy, N., Kirk, L.E., Kersey, J.H., Thomas, E.D., and Balfour, H.H. Jr (1988) Acyclovir for the prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. *N Eng J Med* **318**: 70–75.

Morton, P., and Thomson, A.N. (1989) Oral acyclovir in the treatment of herpes zoster in general practice. *N Z Med J* **102**: 93–95.

O'Brien, J.J., and Campoli-Richards, D.M. (1989) Acyclovir: An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* **37**: 233–309.

Peterslund, N.A. (1988) Management of varicella zoster infections in immunocompetent hosts. *Am J Med* **85** (Suppl 2A): 74–78.

Selby, P., Powles, R.L., Blake, S., Stolle, K., Mbidde, E.K., McElwain, T.J., Hickmott, E., Whiteman, P.D., and Fiddian, A.P. (1984) Amino(hydroxyethoxymethyl)purine: A new well-absorbed prodrug of acyclovir. *Lancet* **ii**: 1428–1430.

Štimac, A., and Kobe, J. (1990) A new synthesis of acyclovir prodrugs. N^2-acetylacyclovir and 6-deoxyacyclovir. *Synthesis* **6**: 461–464.

Welch, C.J., Larsson, A., Ericson, A.C., Oberg, B., Datema, R., and Chattopadhyaya, J. (1985) The chemical synthesis and antiviral properties of an acyclovir-phospholipid conjugate. *Acta Chem Scand* [B] **39**: 47–54.

Wood, M.J., Ogan, P.H., McKendrick, M.W., Care, C.D., McGill, J.I., and Webb, E.M. (1988) Efficacy of oral acyclovir treatment of acute herpes zoster. *Am J Med* **85** (Suppl 2A): 79–83.

Appendix

Elemental Analysis

Compound no.	Analysis	C	H	N	Cl	C	H	N	Cl
		calculated				found			
4	$C_{10}H_{14}N_6O_4 \cdot HCl$	37.69	4.74	26.37	11.12	37.73	4.77	26.29	11.91
19	$C_{14}H_{22}N_6O_4$ ·HCl 7/4 H_2O	41.38	6.57	20.68	8.72	41.30	6.37	20.69	8.85
18	$C_{14}H_{22}N_6O_4$ ·5/4 HCl 11/10 MeOH 1·4 H_2O	42.81	6.70	19.84	10.46	42.82	6.50	19.64	10.48
7	$C_{13}H_{20}N_6O_4S$ ·HCl 11/10 H_2O	37.84	5.67	20.36	8.59 S, 7.77	37.45	5.73	20.16	8.49 S, 7.87
6	$C_{11}H_{16}N_6O_4 \cdot HCl$	39.71	5.15	25.26	10.65	39.54	5.21	25.16	10.58
14	$C_{13}H_{20}N_6O_4$ ·HCl 21/10 H_2O	39.17	6.37	21.08		39.15	6.38	21.10	–
5	$C_{11}H_{16}N_6O_4$ HCL·3/2 H_2O	38.72	5.60	23.36	9.85	38.72	5.59	23.32	9.93
9	$C_{13}H_{20}N_6O_4$ HCl·H_2O	41.22	6.12	22.19	9.36	41.09	6.10	22.12	9.26
21	$C_{13}H_{18}N_6O_4$ 11/10 HCl·H_2O	41.04	5.59	22.09	10.25	40.89	5.50	22.36	10.49
16	$C_{13}H_{20}N_6O_4$ 3/2 HCl·H_2O	39.33	5.97	21.17	–	39.39	5.90	20.98	–
22	$C_{17}H_{20}N_6O_4$ HCl·3/4 H_2O	48.34	5.37	19.90	8.39	48.18	5.08	19.72	8.39
15	$C_{12}H_{20}N_6O_4$ 6/5 HCl·1/4 MeOH ·19/20 H_2O	40.47	6.16	21.37	10.82	40.62	5.98	21.20	10.91
8	$C_{12}H_{16}N_6O_4$ 14/5 $C_2HF_3O_2$ 1/10 H_2O	33.48	3.35	13.31	–	33.38	3.29	13.38	–
13	$C_{12}H_{16}N_6O_4$ 14/5 $C_2HF_3O_2$ 1/5 H_2O	33.39	3.37	13.27	–	33.35	3.39	13.36	–
11	$C_{11}H_{16}N_6O_4$ HCl·2H_2O	35.83	5.74	22.79	9.61	35.92	5.61	22.60	9.62
20	$C_{14}H_{23}N_7O_4$ 18/8 HCl·27/25 H_2O	38.60	6.04	21.34	18.23	38.60	5.98	21.37	18.11
12	$C_{11}H_{16}N_6O_4$ HCl·1/5 I-PrOH	38.53	5.37	24.38	10.28	38.31	5.38	24.16	10.37
17	$C_{14}H_{22}N_6O_4$ 11/10 HCl·2/10 MeOH ·1/2 H_2O	43.19	6.61	21.28	9.88	43.29	6.34	21.11	10.21